database. Twenty-two FDA-approved candidate drugs shifted the transcriptome similarly to ADSC treatment; and are thereby promising for RF treatment. Drug screening revealed that candidates which upregulate lipid metabolism or gluconeogenesis decreased collagen production and/or secretion by TGF-B stimulated fibroblasts.
Conclusions: ADSC transplantation may be an effective treatment for the reversal of RF via metabolic reprogramming. Through pharmacogenomics analysis, we identified FDA approved drugs with potential to be repurposed for the treatment of RF based on their potential to induce metabolic alterations similar to ADSCs. Our data highlights the importance of metabolic dysregulation in the pathogenesis of RF and the importance of targeting these pathways in reversing RF.

28
MUTATIONAL SPECTRUM OF ANAL CANCERS FROM PATIENTS TREATED WITH RADICAL CHEMORADIOTHERAPY
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Purpose: The mutational landscape of anal cancers has not been well studied. The purpose of this study was to perform the first analysis characterizing the types and frequencies of mutations in anal cancers from patients treated with radical chemoradiotherapy (CRT) using comprehensive next-generation sequencing (NGS).
Methods and Materials: Pre-treatment formalin-fixed, paraffin-embedded anal cancer specimens from 30 patients treated with radical CRT for anal cancer at a single tertiary institution were evaluated. Ninety percent of cases were squamous cell cancers. M:F ratio was 1:2.3; median patient age was 56 years (range: 34-80): 47% (n = 14) had T2 disease. Tumour DNA was extracted and assayed for 50 oncogenes and tumour suppressor genes using the Ampliseq Cancer Hot Spot Panel (CHPv2) on the Ion PGM using a 316v2 chip. Mean depth of target coverage was 1005X. Bioinformatic analysis was performed using Torrent Suite Software version 5.03. Variants from reference hg19 were called using variant Caller plugin 5.03.5 and annotated with Ion Reporter Software 5.0. All variants were manually reviewed using Broad Institute's Integrative Genomics Viewer. Mutational status was determined and associated with HPV status.
Results: Twenty-five of 30 cases (83%) were evaluable for full mutational analysis. The most common mutation identified was PIK3CA (4/25 of cases, 16%); 75% (3/4) were in exon 9. Overall, Pi3K/AKT/mTOR pathway activating mutations were seen in 24% (6/25 of cases). Other mutations were very rare: FBXW7 (n = 1, 4%), p53 (n = 1, 4%), IDH1 (n = 1, 4%). One tumour had NRAS mutation; notably all other MAPK pathway genes were wild-type. Twenty-one of 25 cases were HPV sub-typed; 90% (19/21) were positive for high-risk HPV. Only p53 mutation was associated with HPV negative status.
Conclusions: Pi3K/AKT/mTOR activating mutations were the most frequently observed in patients with anal cancer treated with CRT. Anal cancers have targetable mutations, making them amenable for consideration of therapeutics such as Pi3K and EGFR inhibitors. Validation with a larger data set will be undertaken to confirm these findings, and to determine their association with clinical outcome parameters.

29
COMPUTER-ASSISTED IMAGE ANALYSIS OF AN ORAL CAVITY SQUAMOUS CELL CARCINOMA TISSUE MICROARRAY
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Purpose: The immune microenvironment within tumours is critical to oncogenesis, cancer progression, and radiotherapy (RT) efficacy. Immunohistochemistry (IHC) is a convenient and inexpensive method by which to characterize the immune infiltrates in pathology samples. However, manually reading multiple IHC stains on tissue microarrays (TMA) is labor intensive and subject to bias. Our objective is to apply computer image analysis tools to localize and quantify immune markers in oral cavity squamous cell carcinoma (OCSCC) samples and determine their prognostic implications.
Methods and Materials: A 91-patient OCSCC TMA was stained for the markers: CD3, CD4, CD8, FOXP3, IDO, and PD-L1. Tissue Studio (Definiens AG, Munich, Germany) was used to enumerate the number of marker-positive cells and to quantify the staining intensity for IDO and PD-L1. Cell populations were assigned to stromal or epithelial (tumour) compartments according to a mask derived from a pan-cytokeratin stain using a custom Matlab script. Automated methods were validated against manual tissue segmentation, cell count and stain intensity quantification. Univariate associations of cell counts and stain intensities with smoking status, TNM stage, overall survival (OS), and disease-free survival (DFS) were determined.
Results: 80.6% (737/910) of TMA cores were suitable for analysis, 39.8% (35/88) of patients had a known never-smoker history, and 34.1% (31/91) of patients were treated with RT. Comparison of automated to manual tissue segmentation showed good agreement (Kappa coefficient range: 0.61- 0.75). Automated and manual cell counts and stain intensities were highly correlated (Pearson correlation coefficient range: 0.46 - 0.91, $p < 0.001$ for all). Individual cell counts and stain intensities within the stromal, epithelial, or combined compartments did not display significant association with stage, OS or DFS in the set of all patients and in the subset of patients who received RT ($p \geq 0.05$). Compared to never-smokers, current and ex-smokers had an increased density of FOXP3 cells in the epithelial compartment (OR 12.49, $p = 0.06$), and stronger PD-L1 stain intensity in both epithelial and stromal compartments (OR 4.54, $p = 0.08$; OR 6.58, $p = 0.05$); these results were confirmed by manual scores.
Conclusions: Computer-assisted image analysis can be used for robust quantification of cellular populations by IHC. Our automated methods show that current and ex-smokers have higher density of FOXP3 cells in the epithelial compartment and have more intense PD-L1 staining in both epithelial and stromal compartments. This result is validated with manual IHC scoring. This proof-of-principle study demonstrates the utility of computer-assisted image analysis for high-throughput assessment of multiple IHC markers on TMAs, with potential implications for studies on prognostic and predictive biomarkers.

30
EGFR MUTATIONS AND METABOLIC UPTAKE IN ADVANCED NON-SMALL CELL LUNG CANCER
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Purpose: Early studies have suggested a correlation between fluorodeoxyglucose-positron emission tomography (FDG-PET) uptake and epidermal growth factor receptor (EGFR) mutation status in patients with non-small cell lung cancer (NSCLC). Results from these few studies are conflicting and limited by small subject numbers. The purpose of this study was to determine if such a correlation exists in a large population using standardized diagnostic protocols.
Methods and Materials: A retrospective review was conducted of patients with metastatic non-squamous, non-neuroendocrine, NSCLC who had EGFR mutation testing and FDG-PET imaging between March 2010 and March 2012. All patients had FDG-PET imaging at a central facility using the same scanning protocol. Data was collected on the maximum standardized uptake value (SUVmax) of the primary lung tumour. EGFR mutation testing was done at a central lab using a rapid polymerase chain reaction-based detection technique. Patients were divided into EGFR mutation positive (EGFR+) and EGFR wild type (WT) cohorts.

Results: There were 153 patients: 45 (29%) EGFR+ and 108 (71%) EGFR WT. The median age was 66 years (range, 38-88). The population was composed of 38 (25%) Asian ethnicity, 54 (35%) never-smokers, and 97 (63%) female sex. The maximal diameter of the primary tumour on PET imaging was no different between the two EGFR cohorts (mean 4.0 versus 4.0 cm; p = 0.9). The SUVmax ranged from 1.1 to 28.9. There was no difference in SUVmax between EGFR+ and EGFR WT cohorts (mean 10.4 versus 10.6; p = 0.9). There was a significant correlation between larger tumour size and higher SUVmax (r = 0.47; p < 0.01). Median survival was significantly longer for the EGFR+ cohort (28.4 versus 14.0 months; p = 0.02). On multivariate analysis, when accounting for tumour size, SUVmax was not a significant factor for survival (p = 0.8).
Conclusions: In our study there was no correlation between FDG-PET uptake and EGFR mutation status in patients with metastatic NSCLC. The SUVmax cannot be used to predict EGFR mutation status or survival.

31
LESSONS LEARNED IN CONVERTING FROM LDR TO HDRR PENILE BRACHYTHERAPY
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Purpose: Decades of reported experience with low dose rate penile brachytherapy have demonstrated local tumour control rates and penile preservation of 70-85% at 5-10 years with acceptable side effects. Manual source loading is not available in most centres in North America so there is a need to explore the transition to high-dose rate (HDR) automated afterloading for treatment delivery. Dose homogeneity parameters and fractionation schemes need to be developed and validated.
Methods and Materials: Prior experience in interstitial template-based penile brachytherapy using either manually loaded Ir-192 wire or Pulse Dose Rate after-loading was the basis for transitioning to an HDR treatment schema using Varian GammaMed. Templates with 15-18 mm spacing designed for LDR brachytherapy were found to be not ideal for HDR delivery. Spacing was initially 17 mm but was decreased sequentially to 9 mm. A new design of template was created with holes drilled every 3 mm so that inter-plane and inter-needle spacing could be generally 9 mm but increased to 12 around the urethra. Four patients had a 3-plane implant and two patients had 2 planes. 6-13 catheters were used. Fractionation was 4200/12 for three patients, 4500/12, 5300/17 and 3840/12 for one patient each, with fraction sizes of 3.12-3.75 Gy, given bid six hours apart. PTV ranged from 4 to 57 cc, median V100: 96% (88-99), V125 75% (40-98), V150 42% (18-89), V200 16.8% (4.7-32).
Results: From November 2009 to December 2012 six patients with biopsy-proven SCC of the glans penis received HDR interstitial brachytherapy. Age range 33-77, Stage T1:2 and T2: 4, pN0:1, cN0: 5, Grade was MD in five and PD in one. Median follow up is 55 months (31-73). All six patients are NED, although one patient with local and regional failure was salvaged with partial penectomy and left groin dissection. Toxicity was considerable. Five patients experienced painful necrosis with four requiring Hyperbaric oxygen treatment. One of these subsequently had partial penectomy for recurrence; the other four eventually healed. Three patients had severe meatal stenosis, one requiring a temporary suprapubic tube for 10 months (now resolved) and one requiring a permanent perineal urostomy. Two patients remain potent. Toxicity was related to greater needle spacing, larger fraction size, larger PTV volume and excessive inhomogeneity. 9-12 mm is now considered ideal spacing, with fraction size close to 3 Gy, and with V125 ~ 40%, V150~20% and V200 ~5% . The final patient, whose dosimetry followed these parameters, was the only one who was complication free but also had the smallest PTV. Whether following these dosimetric guidelines will permit safe implantation of larger volumes remains to be determined.
Conclusions: HDR penile brachytherapy is effective and can be delivered safely (as evidenced by two recent reports from Sharma et al. and Kellas-Sleczka et al.) but attention must be paid to catheter spacing, fractionation and implant homogeneity parameters.

32
MULTICENTRE CANADIAN EXPERIENCE USING INTRAOPERATIVE PROSTATE BRACHYTHERAPY FOR TREATMENT OF LOW AND INTERMEDIATE-RISK PROSTATE CANCER; AN EVALUATION OF LONG-TERM BIOCHEMICAL RELAPSE-FREE SURVIVAL OUTCOMES
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Purpose: To estimate the rate of biochemical recurrence in prostate cancer treated with intraoperatively planned, low dose rate, prostate brachytherapy using an automated delivery system (IO-LDRB).
Methods and Materials: Patients treated with IO-LDRB as a single modality treatment for low or low-tier intermediate-risk prostate cancer at three Canadian centres between December 1997 and August 2015 were pooled for analysis. Retrospective or prospective databases were maintained at each centre. For this analysis the datasets were amalgamated and analyzed using the R programming language build 3.1.3 (www.r-project.org). Shapiro-Wilks tests of normality, descriptive statistics and Kaplan-Meier survival estimates of biochemical relapse-free survival (bRFS) were employed for analysis.
Results: 3286 patients with a median follow up of 44 months (0.0 - 212.8) and median biochemical follow up of 40.0 months were analyzed. Median age for treated patients was 65 (42-84) years. In these patients, median initial PSA was 5.6 ng/mL (0.03 – 23.8), 2390 (74%) were T1 and 862 (26%) were T2, and initial Gleason Sum was 6 in 2383 (73%) and 7 in 810 (25%). Most patients had low volume disease: median % positive biopsy tissue 5.0% (0.1-90.0), normal gland volumes: median 34.2cc (10.9 – 77.8) and few urinary symptoms: median pre-implant AUA was 5 (0 -33). 387 (11.8%) of patients received hormones for a median of 3.0 months (0.5-32.1) prior to implant. Median seed activity was 0.437 mCi (0.10 – 0.68), D90 was 186.7 Gy (97.0 – 273.0) and V100 was 99.37% (60.52 – 100.0). In follow up, median last PSA value was 0.13 (0.0 – 901.0) and available in 3192 patients. Biochemical failure was observed in 139 patients (5.8%) and median time to failure was 44.0 months (0.0 – 218.8). Five- and 10-year predicted bRFS were 96% and 86%, respectively. Seventy-four deaths were observed from all causes and of those, no death was attributable to prostate cancer.
Conclusions: This is the largest cohort of patients treated with IO-LDRB and demonstrates it to be an effective treatment option for patients with low and low-tier intermediate-risk prostate cancer. Rates of biochemical relapse were low several years post-treatment.

33
BRACHYTHERAPY AS A SOLE TREATMENT MODALITY FOR EARLY ESOPHAGEAL CANCER (EEC)
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Purpose: EEC is a rare disease entity with only a handful of patients diagnosed every year at most large centres treating esophageal cancer. Standard treatments for EEC include endoscopic mucosal resection, surgery (S) or chemoradiation (CRT). Patients are often not candidate for S or CRT because of their comorbidities or for EMR because of extent of tumour. Brachytherapy in these instances can give high doses of RT locally to the tumour. We present our experience using Radical Brachytherapy (RBT) alone in EEC.